ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Assessment of anti-diabetic activity of alcoholic and hydro-alcoholic extract of *Terminalia arjuna* & *Syzygium cumini* in streptozotocin-induced diabetes in Wistar rats

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Article History:

Received on: 24 Aug 2020
Revised on: 29 Oct 2020
Accepted on: 09 Oct 2020

Keywords:

Diabetes,
Terminalia arjuna,
Syzygium cumini,
Streptozocin. β-cell

ABSTRACT

We aimed to evaluate the effect of anti-diabetic activity of *Terminalia arjuna*, and *Syzygium cumini* extracts in Streptozotocin (STZ) induced diabetes in Wistar rats. STZ (55mg/kg) followed by nicotinamide (100mg/kg) was given to rats by intraperitoneal route to induce diabetes. Oral administration of alcoholic and hydro-alcoholic extracts of *T. arjuna* (TAAE) (250mg/kg and 500mg/kg), *S. cumini* (SCAE) (200mg/kg and 400mg/kg) and their composite extract were given to rats along with standard anti-diabetic drug Glibenclamide (5mg/kg). We evaluated body weight, glucose level, lipid profile and biochemical parameters in STZ induced diabetic rats. Also, histopathological studied were done in liver, kidney and pancreatic tissues of rats. Our finding revealed that TAAE and TAHE at 250mg/kg b.w. and 500mg/kg b.w., SCAE and SCHE at 400mg/kg b.w. and combination of TAAE (250mg/kg b.w.)+SCAE (400mg/kg b.w.) had a positive effect in lowering the blood glucose level and body weight on 28^{th} day as compared to the initial observation on 0^{th} day and also restored all the biochemical parameters such as LDL, VLDL, triglycerides and total Cholesterol and HDL towards the normal levels as well as histopathological improvement in Kidney, Liver and Pancreas. Data analysis showed that composite extract of TAAE (250mg/kg) and SCAE (400mg/kg) improved diabetic consequences more effectively than composite extract of TAHE (500mg/kg) and SCHE (400mg/kg). TAAE and SCHE, in combination, demonstrate as a potential therapeutic agent against diabetes.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4392

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INTRODUCTION

Diabetes Mellitus (DM) is a well-known metabolic disorder, characterised by disturbance in insulin resistance, insulin activity, or both (Kharroubi and Darwish, 2015). Type 1 diabetes (insulin deficiency) and Type 2 diabetes (insulin resistance) are the two major subgroups of diabetes mellitus. Gestational diabetes mellitus (GDM) is one of the commonest metabolic disorder of pregnancy which means high plasma glucose first identified during pregnancy is basic to the consideration of pregnant women (Mukeshagarwal, 2015).

Diabetic microvascular complications such as neuropathy, nephropathy and retinopathy are the significant initiators of chronic hyperglycemia (Sheetz, 2002). It has been demonstrated that oxidative stress and impaired antioxidant defence systems play a significant role in the pathogenesis of diabetes mellitus. There is clinical proof that responsive oxygen species harms pancreatic cells, initiates lipid peroxidation and elevated glucose concentration and in diabetes (Leijin *et al.*, 2008).

Clinically approved anti-diabetic drugs such as biguanides, sulfonylurea and thiazolidinediones are well known to treat hyperglycemia in diabetes Mellitus along with various serious side effects (Kirupa and Kavitha, 2013; Aiman, 1970), so it's a challenge to combat side effects with potential therapeutic effect for the management of diabetes. Thus, treatment of diabetes has moved to common plants; it is likewise referenced that herbal drugs may show a synergistic or antagonist effect when used in combination (Saxena and Vikram, 2004).

Terminalia arjuna (*T. arjuna*, Family: Combretaceae), is an important medicinal plant widely used in medicinal formulations for several ailments such as high cholesterol, diabetes, heart diseases like chest pain and heart failure etc. It is found in abundance throughout Indo-sub-Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi and Deccan region near ponds and rivers (India) as well as found in forests of Sri Lanka, Burma and Mauritius (Khaliq and Fahim, 2018).

Syzygium cumini (Linn.) Skeels (Myrtaceae) usually known as Indian blackberry; Jamun, is a huge tree disseminated all through Upper Gangetic Fields, Bihar, Orissa, planted in West Bengal, Deccan, Konkan area; additionally, developed in Thailand, Philippines, Madagascar and developed broadly all through Africa, Caribbean and Tropical America (Kumar *et al.*, 2019). This medicinal plant has a therapeutic effect such as anti-inflammatory, anti-bacterial anti-bacterial, antioxidants, anti-hyperlipidemic, anti-hypertensives etc.

Many works have been done in *T.arjuna*, and *S. cumini* on Diabetes mellitus like both plants of extracts possess anti-diabetic effect (Kumar *et al.*, 2008; Morshed *et al.*, 2011). Both the selected plants have a different mode of action, and there is no evidence of anti-diabetic effect when used in combination. Therefore, we focussed on analysing the efficacy of composite extract of *Terminalia arjuna and Syzygium cumini* in STZ induced diabetes in rats, If this combination shows a positive effect, it would be a noble combination for treating diabetic patients.

MATERIALS AND METHODS

Preparation of alcoholic and hydro-alcoholic extract

The Stem bark of *Terminalia arjuna* and seeds of *Syzygium cumini* were purchased from Khari Baoli, New Delhi, India and identified by the National Institute of Science Communication and Information Resources (NISCAIR), Delhi, India with Ref.No: NISCAIR/RHMD/Consult/2018/3267-68-1 of Terminalia arjuna and Ref.No: NISCAIR/RHMD/Consult/2018/3267-68-1of *Syzygium cumini.* The powdered drug of *Terminalia arjuna* and *Syzygium cumini* was extracted with Alcohol(Ethanol 90%) and Hydro-alcoholic (Ethanol 50% and Water 50%) solution using the Soxhlet method (Mishra *et al.*, 2014). The extracts were filtered separately and evaporated to dryness to yield the dry extracts and kept in a vacuum desiccator until use. A crude residue (150g) of *Terminalia arjuna* and *Syzygium cumini* were obtained, giving a yield of 7.90% and 4.78% (alcoholic) and 7.50% and 6.90% (Hydroalcoholic).

Animals

Wistar rats of either sex (6 -7-week-old) weighing 200-250g were used and kept in the Animal House Facility, KIET School of Pharmacy, Ghaziabad. The animals were placed in polypropylene cage under standard laboratory condition (12 h light and 12 h dark cycle). They had free access to commercial pellets diet (Pranave Agro Industries Ltd, New Delhi) and water ad *libitum.* The animal house temperature was maintained at 25 $\pm$ 2ºC. The protocol was approved from the Institutional Animal Ethics Committee (IAEC) of KIET School of Pharmacy (IAEC/KSOP/E/18/09) Ghaziabad.

Streptozotocin diabetes model

Intraperitoneal injection of 55mg/kg b.w of STZ was given to rats followed by, administration of nicotinamide 15 minutes later (120mg/kg) to induce diabetes in rats. After 3-4 days, we measured fasting blood glucose level (FBG) of rats, and it was found in the range of 180 and 220mg/dL, which comes in the diabetic range. These diabetic rats were additionally grouped (n = 6) and were given test and standards for 15 days as indicated by the accompanying convention (Nayak *et al.*, 2014).

Experimental design

Rats of either sex were divided into 13 groups containing 6 animals each - Group-I Rats treated with only normal saline; Group-II STZ treated (55mg/kg/b.w) +Nicotinamide (100mg/kg/b.w); Group-III Glibenclamide (5mg/kg/b.w); Group-

IV TAAE Low Dose (250mg/kg/b.w); Group-V TAAE High Dose(500mg/kg/b.w); Group-VI TAHE Low Dose(250mg/kg/b.w); Group-VII TAHE High Dose(500mg/kg/b.w); Group-VIII SCAE Low Dose(200mg/kg/b.w); Group- IX SCAE High Dose(400mg/kg/b.w); Group-X SCHE Low Dose(200mg/kg/b.w); Group- XI SCHE High Dose(400mg/kg/b.w); Group-XII TAAE + SCAE; Group- XIII TAHE + SCHE. Treatment was given for 28 days.

Bodyweight measurement

Bodyweight of the rat was measured on 0 days, 7^{th} day, 14^{th} day, 21st day and 28^{th} day by using a weighing balance (Ekambaram *et al.*, 2010).

Blood glucose estimation

A blood sample was collected from the tail vein of rats and measured blood glucose level by using Dr Morepen glucometer as per instructions of producer (Kumar *et al.*, 2013).

Collection of blood and tissue

By using mild chloroform anaesthesia, the animals were euthanised by cervical dislocation at the end of the treatment period. Serum was separated by centrifugation after collecting the blood on decapitation. The tissues and serum were collected and used for biochemical and histochemical analysis.

Estimation of Biochemical Parameters

High-density lipoprotein (HDL)- Cholesterol measurement

HDL cholesterol was estimated by Phosphotungstic acid method by using the respective kit(Erba Mannheim Germany Kit), as per manufacturer's protocol. HDL cholesterol amount was calculated by using the formula given below:

$$\text{HDL cholestrol}\left(\frac{mg}{dl}\right) = \text{Abs. of}\frac{\text{Test}}{\text{Standard}} \times \text{Concentration of standard}\left(\frac{mg}{dl}\right) \times \text{Dilution factor}$$

Albumin level measurement

Albumin level was measured by BCG Dye method by using the respective kit (Erba Mannheim Germany Kit), as per manufacturer's protocol. Albumin level was calculated by using the formula given below:

$$\text{Albumin}\left(\frac{g}{dl}\right) = \text{Abs. of}\frac{\text{Test}}{\text{Standard}} \times \text{Concentration of standard}\left(\frac{g}{dl}\right)$$

Bilirubin level estimation

Bilirubin (Bit and Bid) level was measured by Diazo method by using the respective kit (Erba Mannheim Germany Kit), as per manufacturer's protocol. Bilirubin level was calculated by using the formula given below:

$$\text{Total Bilirubin}\left(\frac{mg}{dl}\right) = \text{Abs. of}\frac{\text{Test}}{\text{Standard}} \times \text{Concentration of standard}\left(\frac{mg}{dl}\right)$$

Alkaline Phosphate (ALP) level measurement

Alkaline phosphate was detected by IFCC (International federation of clinical chemistry) method, and Kinetic was measured by using the respective kit(Erba Mannheim Germany Kit), as per manufacturer's protocol and calculated by using the formula given below:

$$\text{ALP activity}\left(\frac{IU}{L}\right) = \frac{\triangle \text{A}}{min.} \times \text{Factor}\ (2764)$$

Serum Glutamic Oxaloacetic Transaminase and Serum Glutamic Pyruvic Transaminase (SGOT and SGPT) level measurement

SGOT and SGPT level was measured by IFCC (International federation of clinical chemistry) method, and Kinetic was measured by using the respective kit(Erba Mannheim Germany Kit), as per manufacturer's protocol and calculated by using the formula given below:

$$\text{Activity of AST} = \frac{\triangle \text{Abs}}{\text{min}.} \times 1768$$

Histopathology

The pancreas, kidney and liver were preserved in 10% formalin immediately after extracted from the animals.

Statistical analysis

Statistical evaluation was analysed by using Graphpad Prism. One-way ANOVA, followed by Dunnett's multiple comparison test, was used for statistical comparison. P values of < 0.05 were observed as statistically significant.

RESULTS

Effect of alcoholic and hydro-alcoholic extracts on body weight in rats

The body weight of rats from STZ induced positive control group (after 28 days) was significantly (P < 0.05) reduced as compared to control and standard groups. Oral administration of TAAE at 250mg/kg b.w. and 500mg/kg b.w. significantly (P < 0.05) improved as compared with positive control. TAHE at 250mg/kg b.w. significantly (P < 0.05) improved body weight at $21^{st}$and 28^{th}daytime point while the improvement was seen to be on 14^{th}, 21^{st} and 28^{th} days respectively at 500mg/kg b.w. which was approximately equal to standard diabetic group. SCAE at 200mg/kg b.w. and 400mg/kg b.w. significantly (P < 0.05) improved body weight on 14^{th}, 21^{st} and 28^{th} days, respectively. SCHE at 400mg/kg b.w. significantly (P < 0.05) improved, which was almost equal to the standard diabetic group. Combination dose of alcoholic TAAE (LD) + SCAE

(HD)significantly (P < 0.05) improved body weight which was equal as compared to the standard diabetic group. Combination dose of hydro-alcoholic TAHE (HD) + SCHE (HD)significantly (P < 0.05) improved the body weight observed on 14^{th}, 21^{st} and 28^{th} days, respectively. Body weight changes among different groups are shown in Table 1 and Figure 1.

Effect of Alcoholic and Hydro-alcoholic extracts on glucose levels

The glucose level of STZ induced positive control group rat (after 28 days) was significantly (P < 0.05) elevated as compared with control and standard group. Oral administration of TAAE at 250mg/kg b.w. significantly (P < 0.05) reduced glucose level as compared with positive control and at 500mg/kg b.w. glucose level reduction was observed on 21^{st} and 28^{th} days, respectively. TAHE at 500mg/kg b.w. significantly (P < 0.05) reduced, which was equal as compared to the standard diabetic group. SCAE at 200mg/kg b.w. did not affect glucose level and at 400mg/kg b.w. Significantly (P < 0.05) reduced glucose level when compared with the positive control group. SCHE at 200mg/kg b.w. significantly (P < 0.05)decrease glucose level observed on 7^{th}, 14^{th} and 28^{th} day only and at 400mg/kg b.w., Significantly (P < 0.05) reduced glucose level which was equal as compared to standard diabetic group. Combination dose of alcoholic TAAE (LD) + SCAE (HD)significantly (P < 0.05) decreased glucose level, which was equal as compared to the standard diabetic group. Combination dose of Hydro-alcoholic TAHE (HD) + SCHE (HD)significantly (P < 0.05) reduced glucose level. Changes in blood glucose level are shown in Table 2 and Figure 2 among different groups.

Biochemical profile of alcoholic and hydro-alcoholic extracts

Effect of alcoholic and hydro-alcoholic extracts on lipid profile and enzymatic parameters are mentioned below:

Lipid profile

STZ induced positive control group significantly (P < 0.05) increased Total cholesterol, Triglycerides, Low-density lipoprotein & Very low-density lipoprotein while decreased High-density lipoprotein level as compared to the control group. Oral administration of TAAE (250mg/kg and 500mg/kg)significantly (P < 0.05) decreased the level of TC, TG, LDL, VLDL and raised the level of HDL as compared with positive control. However, TAHE (250mg/kg)did not show any significant effect on lipid profile but at 500mg/kg dose significantly (P < 0.05) decreased the level of TC, TG, LDL, VLDL and increased HDL level. SCAE and SCHE at 200mg/kg b.w. did not produce significant changes on lipid profile but at 400mg/kg b.w., significantly (P < 0.05) reduced the level of TC, TG, LDL, VLDL and raised the level of HDL. Combination dose of alcoholic TAAE (LD) and SCAE (HD)significantly (P < 0.05) decreased the level of TC, TG, LDL, VLDL and increased the level of HDL as compared to a standard diabetic group, while combination dose of hydro-alcoholic TAHE (HD) and SCHE (HD)did not produce a significant effect on lipid profile as shown in Table 3 and Figure 3.

Enzymatic parameters

Statistical analysis showed that STZ induced positive control group significantly (P < 0.05) elevated the level of albumin, bilirubin, alkaline phosphate, alanine transaminase, and aspartate transaminase compared to control group. Oral administration of TAAE and TAHE at 250mg/kg b.w. and 500mg/kg b.w. dose significantly (P < 0.05) improved the level of albumin, bilirubin, ALP, ALT and AST. SCAE and SCHE at 400mg/kg b.w. significantly (P < 0.05) reduced the level of albumin, bilirubin, ALP, ALT and AST when compared with the positive control group. Combination dose of alcoholic TAAE (LD) and SCAE (HD)significantly (P < 0.05) reduced the level of albumin, bilirubin, ALP, ALT and AST. A similar effect was shown in the group given with the combination dose of hydro-alcoholic TAHE (HD) and SCHE (HD)as shown in Table 4 and Figure 4.

Histopathology of liver, kidney and pancreas

Liver

Histopathological study of the liver showed necrosis and fibrotic changes with conspicuous evidence of fatty deposits in the diabetic liver when compared with normal liver showing the central vein with radiating cords of hepatocytes. Standard group showed normal portal tract (PT) and TAAE at 250mg/kg b.w. showed the evidence of swelling and fat deposits and 500mg/kg b.w. showed minor necrosis and fibrotic changes. TAHE at 250mg/kg b.w. also showed minor necrosis and fibrotic changes, while at500mg/kg b.w. showed the evidence of hypertrophy and disarrangement of hepatic parenchyma**.** SCAE at 200mg/kg b.w**.** showed cell swelling and congestion, while at 400mg/kg b.w. showed portal veins with mild haemorrhage. SCHE at 200mg/kg b.w. showed normal features and 400mg/kg b.w. showed minor necrosis and fibrotic changes. Combination dose of alcoholic TAAE (LD) and SCAE (HD)showed normal cells like the diabetic group. In contrast, a combination dose of hydro-alcoholic TAHE (HD) and SCHE (HD)showed mild swelling

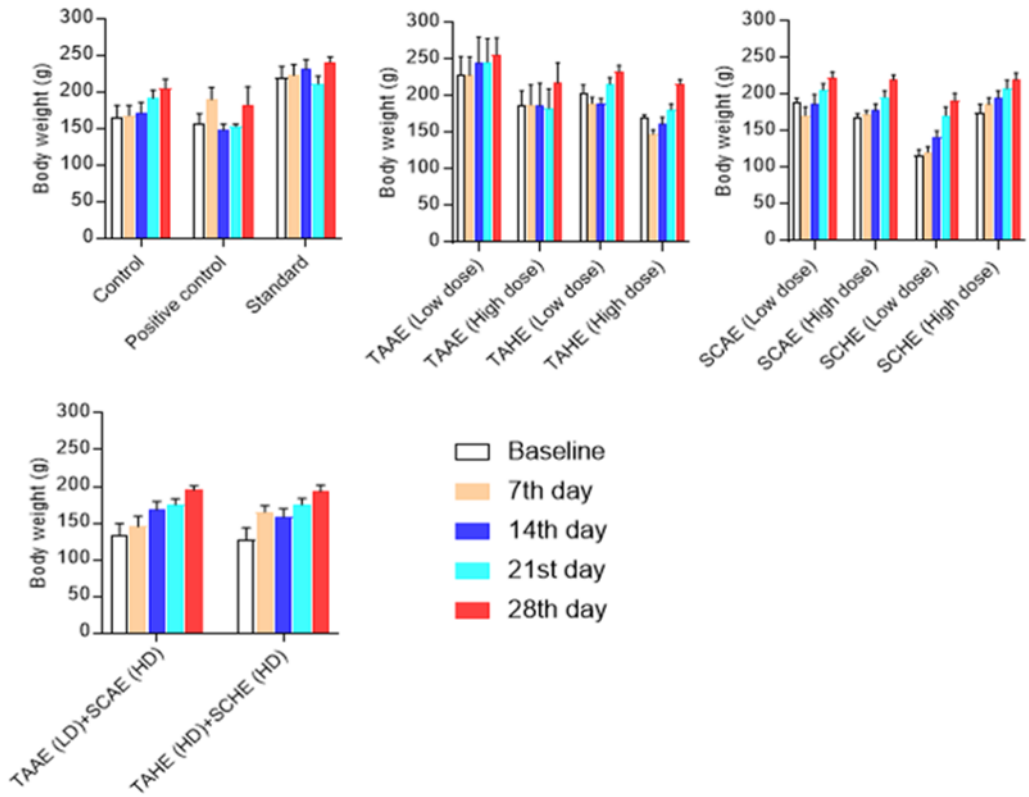

Figure 1: Body weight measurement. Bar diagram representing changes in body weight.

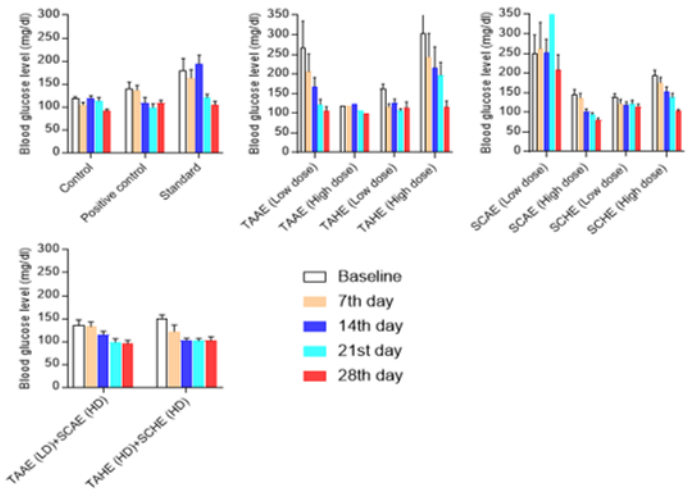

Figure 2: Blood glucose level estimation. Bar diagram representing changes in blood glucose level.

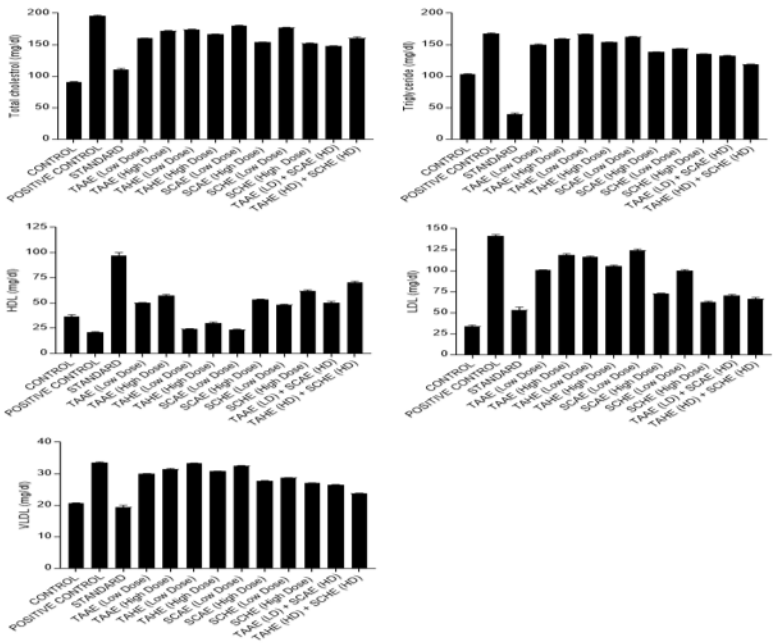

Figure 3: Lipid profile assessment. Bar diagram representing changes in lipid profile.

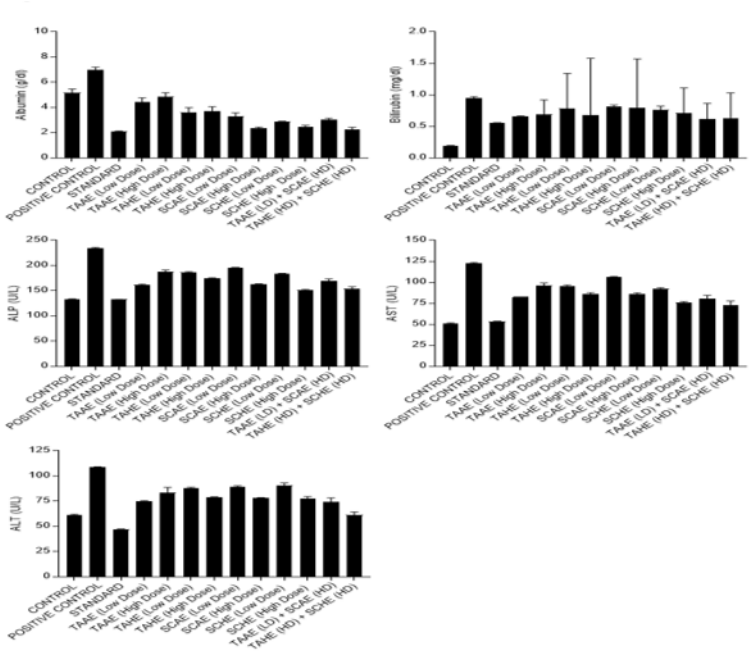

Figure 4: Liver enzymatic activity. Bar diagram representing changes in liver enzymatic activity.

on hepatic cells as the evidence of hypertrophy, as shown in Figure 5.

Kidney

In the histopathology of the kidney, we found that the diabetic group had necrosis and damage epithelium cells and proteinuria when compared with normal liver showing proximal convoluted tubules and glomeruli. The standard group showed degeneration in tubular epithelium cells and deterioration of the glomerular structure. TAAE at 250mg/kg b.w. showed thickening of renal vesicles and fibrotic changes of the glomeruli, but 500mg/kg b.w. group rate stored cellular integrity and reversed glomeruli atrophy.TAHE at 250mg/kg b.w also showed degeneration and deterioration of glomerular structure but 500mg/kg b.w. showed mild necrosis and damaged epithelium cells. SCAE at 200mg/kg b.w.showed restoration of cellular integrity and reversed glomeruli atrophy but at 400mg/kg b.w.showed degeneration and deterioration of glomerular structure. In SCHE at 200mg/kg b.w.group rats, some glomerulus had dilated loops

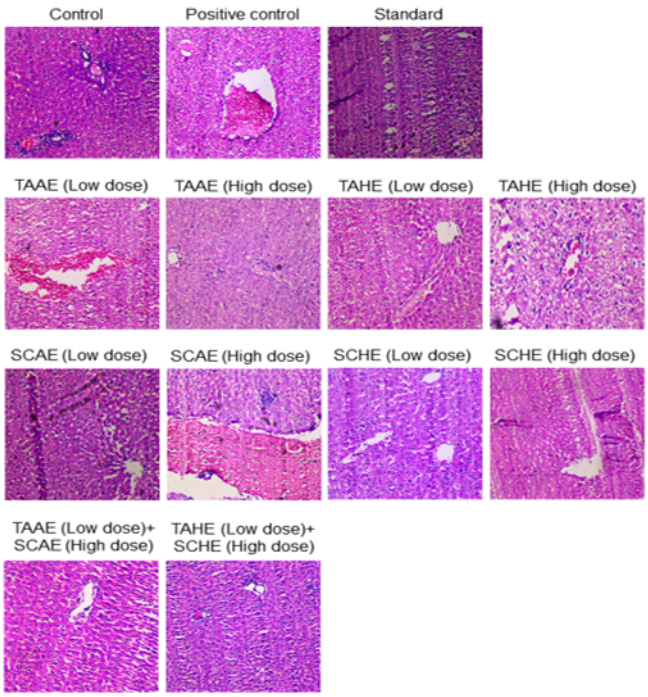

Figure 5: Histopathological changes in the liver. Representative images were showing changes in rat liver tissue.

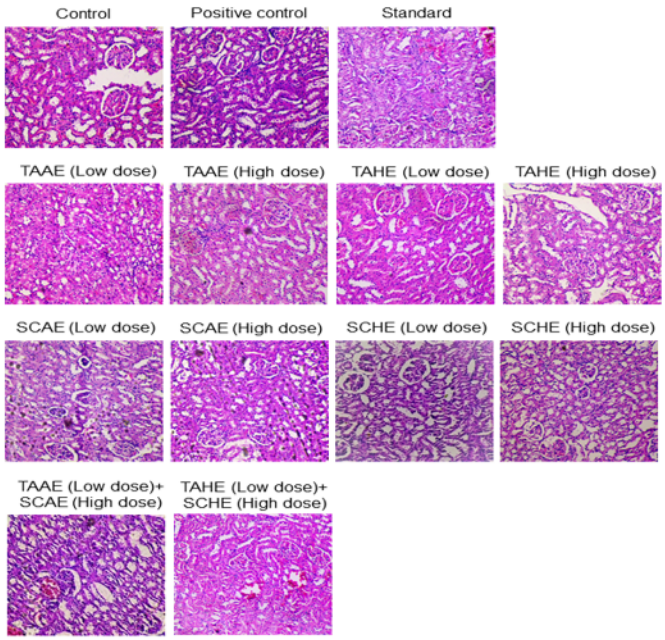

Figure 6: Histopathological changes in the kidney. Representative images are showing changes in rat kidney tissue.

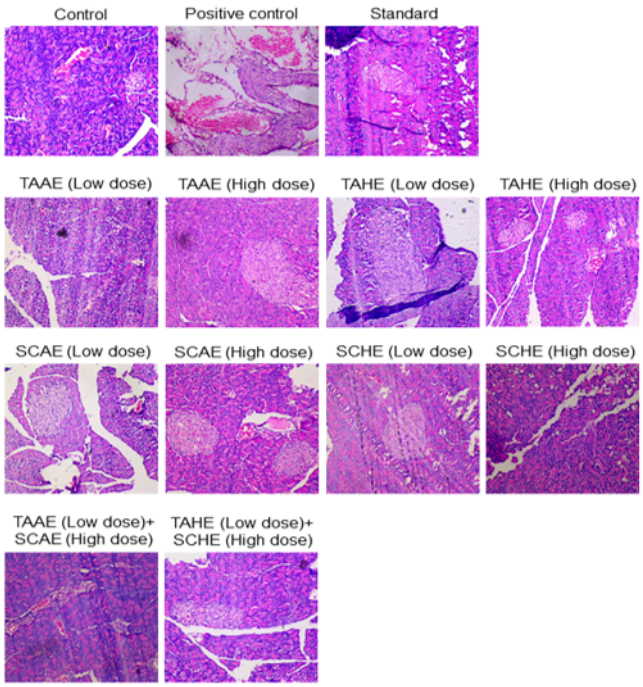

Figure 7: Histopathological changes in the pancreas. Representative images showing changes in rat pancreas tissue.

Table 1: Effect of all doses with combinations on body weight along with Control, Positive Control, Standard

Group Description	Days				
	0th	7th	14th	21st	28th
Control	165.00±17.07	168.33±14.00	171.66±14.92	191.67±11.66	205.00±12.84
Positive Control	158.33±12.49	190.00±16.53	148.33±8.33	153.33±3.33	183.33±24.17
Standard	220.00±15.05	223.33±14.53	231.66±13.01**	211.66±10.46*	241.66±6.50*
TAAE(Low Dose)	228.33±25.87	228.33±25.22	245.00±35.53**	246.66±31.69**	256.66±22.90**
TAAE(High Dose)	188.33±18.87	188.33±27.13	186.67±30.94	183.33±26.41	218.33±27.13
TAHE (Low Dose)	205.00±10.56	190.00±8.56	190.00±6.83	216.66±8.43	233.33±8.43
TAHE(High Dose)	170.00±4.47	148.33±6.00	161.66±9.45	181.66±7.49*	216.66±6.14
SCAE(Low Dose)	190.00±5.16	171.66±11.66	186.66±13.58	206.66±8.43	223.33±7.60
SCAE(High Dose)	168.33±5.42	173.33±4.94	178.33±8.72	196.66±8.02	220.00±6.83
SCHE(Low Dose)	116.66±8.02	121.66±7.03*	141.66±8.72	171.66±11.37	191.66±10.13
SCHE(High Dose)	175.00±11.76	186.66±9.18	195.00±9.91	208.33±11.08	220.00±9.66
TAAE(LD)+SCAE(HD)	133.33±16.05	145.00±14.08	168.33±11.37	175.00±8.46	195.00±6.19##
TAHE(HD)+SCHE(HD)	128.33±15.14	165.00±8.85	158.33±11.66	175.00±8.85	193.33±8.81###

Results were expressed Mean ± SEM (n=6) P < 0.05; *P < 0.05 versus Positive control, ** P < 0.005 versus Positive Control, ##P< 0.002 versus Standard, ###P < 0.005 versus Standard.

Table 2: Effect of all doses with combinations on glucose level along with Control, Positive Control, Standard

Group Description	Days				
	0th	7th	14th	21st	28th
Control	118.16±4.02	104.83±5.10	118.66±5.81	113.16±7.17	91.00±3.95
Positive Control	140.83±13.08	136.66±10.50	108.50±11.85*	98.66±8.26	108.66±5.85
Standard	180.33±25.45	162.83±18.22	193.33±19.42	120.00±7.95	104.83±7.05
TAAE(Low Dose)	266.83±68.69	205.83±45.74	168.66±22.33	122.66±13.32	106.33±10.63
TAAE(High Dose)	119.83±00	118.66±00	123.66±00	107.00±00	100.50±00
TAHE(Low Dose)	163.16±11.51	116.83±7.77	126.83±9.88	107.16±4.71	113.50±15.49
TAHE(High Dose)	304.16±68.58	244.16±59.43	215.16±54.29**	197.00±33.01*	115.83±15.91
SCAE(Low Dose)	250.16±47.22	262.16±66.9*	252.33±34.31****	460.50±65.85****	208.00±39.03
SCAE(High Dose)	145.00±13.29	137.00±11.57	102.16±6.36	94.33±4.69	80.33±4.93
SCHE(Low Dose)	137.83±9.17	122.83±10.50	118.00±9.02	121.50±9.41	115.33±5.71
SCHE(High Dose)	193.66±13.78	175.83±13.71	154.33±10.60	137.83±10.98	103.83±3.82
TAAE(LD)+SCAE(HD)	135.66±11.53	132.50±10.21	114.50±7.73	98.66±7.56	95.00±7.58
TAHE(HD)+SCHE(HD)	151.16±7.29	121.83±14.17	102.33±5.09	101.66±5.06	101.83±8.67

Results were expressed Mean ± SEM (n=6) P< 0.05; *P < 0.05, **P < 0.005, ****P< 0.0001

Table 3: Effect of all doses with combinations on Lipid Profile of streptozotocin-induced diabetic rats along with Control, Positive control, Standard after 28 days

Group Description	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	91.16±0.47	103.16±1.06	36.83±1.42	33.7±1.50	20.63±0.14
Positive Control	195.83±1.30	167.66±1.45	20.83±0.79	141.46±1.77	33.53±.0.29
Standard	110.0±2.35****	40.16±1.35****	96.83±3.34****	53.33±3.36****	19.36±0.66****
TAAE (Low Dose)	160.0±0.57****	150.50±0.76****	50.0±0.57****	100.50±0.65****	30.0±0.115****
TAAE (High Dose)	172.16±0.67****	159.50±0.42****	57.66±0.88	119.13±1.16****	31.53±0.17****
TAHE (Low Dose)	174.33±0.33****	166.50±0.56	24.16±0.47	116.86±0.69****	33.30±0.11
TAHE (High Dose)	166.66±0.42****	153.83±0.60****	30.33±0.91****	105.56±1.16****	30.76±0.12****
SCAE (Low Dose)	180.66±0.66****	162.83±0.54***	23.66±0.61	124.43±1.21****	32.56±0.10*
SCAE (High Dose)	154.00±0.36****	138.33±0.47****	53.50±0.42****	72.73±0.63****	27.76±0.09****
SCHE (Low Dose)	177.33±0.66****	143.50±0.56****	48.33±0.42****	100.30±0.96****	28.70±0.11****
SCHE (High Dose)	152.16±0.87****	135.16±0.47****	62.16±0.86****	62.96±0.86****	27.03±0.09****
TAAE(LD) + SCAE(HD)	147.66±0.80****	132.66±0.55****	50.50±1.25****	70.63±1.24****	26.50±0.11
TAHE(HD) + SCHE(HD)	160.66±1.74****	119.00±0.36****	70.66±0.91****	66.20±1.95****	23.80±0.07****

Results were expressed Mean ± SEM (n=6) P < 0.05; *P < 0.05, ***P < 0.004, ****P <0.0001

Table 4: Effect of all doses with combinations on Liver functions of streptozotocin-induced diabetic rats along with Control, Positive control, Standard after 28 days

Group Description	Albumin (g/dL)	Bilirubin (mg/dL)	ALP (U/L)	AST (U/L)	ALT (U/L)
Control	5.16±0.30	0.187±0.01	133.66±0.42	51.36±0.43	61.55±0.46
Positive Control	7.00±0.20	0.952±0.018	234.66±1.20	122.83±0.74	108.58±0.55
Standard	2.11±0.03****	0.55±0.009****	132.50±0.56****	53.33±0.42****	46.83±0.60****
TAAE (Low Dose)	4.41±0.35****	0.66±0.008****	162.00±1.12****	82.50±0.22****	75.16±0.30****
TAAE (High Dose)	4.85±0.32****	0.69±0.23****	187.83±3.20****	96.16±3.06****	83.66±5.07****
TAHE (Low Dose)	3.61±0.38****	0.78±0.56****	186.16±1.55****	95.66±1.14****	88.33±0.55****
TAHE (High Dose)	3.73±0.33****	0.68±0.90****	174.76±0.96****	86.33±1.40****	79.00±0.25***
SCAE (Low Dose)	3.30±0.27****	0.82±0.02***	195.91±0.29****	106.16±0.87****	89.38±0.85****
SCAE (High Dose)	2.36±0.08****	0.79±0.78****	162.33±1.40****	86.00±1.52****	78.16±0.47****
SCHE (Low Dose)	2.89±0.019****	0.76±0.06****	183.16±1.55****	92.50±1.11****	90.83±2.37****
SCHE (High Dose)	2.44±0.14****	0.71±0.4****	151.83±0.87****	75.83±1.40****	77.83±1.71****
TAAE (LD) + SCAE (HD)	3.05±0.09****	0.615±0.25****	168.83±4.56****	80.66±3.97****	74.00±4.29****
TAHE (HD) + SCHE (HD)	2.24±0.18****	0.63±0.40****	153.66±4.16****	73.00±4.81****	61.33±2.97***

Results were expressed Mean ± SEM (n=6) P < 0.05; ***P < 0.0002, ****P < 0.0001

and 400mg/kg b.w.ratsshowed thickening of renal vesicles and fibrotic changes of the glomeruli. Combination dose of alcoholic TAAE (LD) and SCAE (HD) restored cellular integrity. It reversed glomeruli atrophy, while combination dose of hydro-alcoholic TAHE (HD) and SCHE (HD) group glomerulus had suffused RBC's with mild necrosis of epithelium cells as shown in Figure 6.

Pancreas

Histopathology of pancreas showed damaged pancreatic islets cells in the diabetic group when compared with normal pancreas showing exocrine and endocrine cells. The standard group showed exocrine acinus and preserved islets of Langerhans with normal β-cell population. TAAE at 250mg/kg b.w. showed shrunken and distorted islets of Langerhans and 500mg/kg b.w. showed a mild increase in the size of islets of Langerhans. TAHE at 250mg/kg b.w. showed an increase in the size of islets of Langerhans and at 500mg/kg b.w. showed degeneration of islets and necrotic changes. SCAE at 200mg/kg b.w. showed preserved islets of Langerhans, while at 400mg/kg b.w. showed mild fibrosis and inflammatory cell infiltration into the islets of Langerhans. SCHE at 200mg/kg showed normal pancreatic islets, while at 400mg/kg b.w. showed exocrine acinus and small preserved islets. A combinational dose of alcoholic TAAE (LD) and SCAE (HD)showed a moderate decrease in the number of islets of langer hans and β-cells and combination dose of hydro-alcoholic TAHE (HD) and SCHE (HD)showed a mild decrease in the number of islets of langerhans and β-cells as shown in Figure 7.

DISCUSSION

Alloxan and STZ are the most widely recognised substances in inducing diabetes in the rodents or rats, which take pancreatic β-cells by means of glucose transporter GLUT2. The nitrosourea moiety of STZ induced β-cell death via DNA alkylation, which initiates the process of nitric oxide(NO) and receptive oxygen species production (Vessal *et al.*, 2003). Exhaustion of intracellular nicotinamide dinucleotide (NAD) in islet cells and methylation in pan-

creatic islet and DNA strand breaks cells are the most activities occurred following STZ administration (Coskun *et al.*, 2005).

In support of these findings, data TAHE at 500mg/kg b.w., SCAE and SCHE at 400mg/kg b.w., a combination of TAAE (250mg/kg b.w.) and SCAE (400mg/kg b.w.) significantly ($P < 0.05$) improved body weight, normalised the blood glucose level and restored the lipid profile as well as liver biochemical parameters towards the normal range. Our finding revealed that TAAE and TAHE at 250mg/kg b.w. and 500mg/kg b.w., SCAE and SCHE at 400mg/kg b.w. and combination of TAAE (250mg/kg b.w.)+SCAE (400mg/kg b.w.) had a positive effect in lowering the blood glucose level and body weight on 28^{th} day as compared to the initial observation on 0^{th} day.

Treatment of *T. arjuna bark* to the STZ induced diabetic rats reduced the glucose level and raised the insulin level due to inhibition of insulin input and intestinal glucose absorption. The extracts may have the abilities to rejuvenate the secretion of the insulin for β-cell by several mechanisms and the most effective approach for controlling DM and improved carbohydrate analysis of this present study revealed that the following groups such as TAAE at 250mg/kg b.w., metabolism (Kumar *et al.*, 2013). *Terminalia arjuna* contains active principles like glycosides, terpenoids, flavonoids, arjunic acids etc. possessing anti-diabetic activity (Barman and Das, 2012).

Concentrate of *T. arjuna* was found to be more powerful than allopathic medication or firmly viable when contrasted with metformin, thus established an extremely quick recovery from diabetic status by treatment with herbal extract of *Terminalia arjuna* and metformin (Aparajita *et al.*, 2018). The homoeopathic mother tincture of *S. cumini* recuperate the levels of glycogen in the liver and skeletal muscle tissues and expanding the levels of serum urea, uric acid and creatinine also recuperate the activities of antioxidant enzymes like catalase, peroxidase and superoxide dismutase (Ghosh *et al.*, 2014). The extract of *S. cumin* also showed expanded α-amylase inhibitory effect up to 95.4%, therefore, a significant amount of flavonoid in the seed of *Syzygium cumini* contributes for anti-diabetic activity (Prabakaran and Shanmugave, 2018).

In our study, alteration in lipid profile such as LDL, VLDL, triglycerides and total Cholesterol and HDL was observed on 28^{th} days in diabetic rats when compared to treatment and standard group. Elevated cholesterol, LDL, VLDL and HDL were normalized in diabetic rats after the treatment of TAAE and TAHE at 250mg/kg b.w. and 500mg/kg b.w., SCAE and SCHE at 400mg/kg b.w. and combination of TAAE (250mg/kg b.w.) and SCAE (400mg/kg b.w.). The HMG-CoA reductase, glucose-6-phosphate dehydrogenase, malate dehydrogenase of activity was decreased. It showed the enhanced rate of degradative processes and reduction in intestinal absorption of free cholesterol and other lipids in the high concentrations of faecal neutral sterols and bile acids in the liver (Patil *et al.*, 2011).

The clearance of hepatic cholesterol was increased due to *S. cumini* seeds being shipped from the extra-hepatic tissues. Extract of seed *Syzygium cumini* decrease not only the level of triglycerides and total cholesterol but also the restores the function of hepatic specific enzymes by inhibition of oxidative stress (Hossain *et al.*, 2011). Elevation of renal enzymes such as SGOT, SGPT, ALP, albumin and bilirubin was also observed in diabetic rats pointed to tainted liver function due to hepatic damage. (Biswas *et al.*, 2011). Twenty eight days treatment with TAAE and TAHE at 250mg/kg b.w. and 500mg/kg b.w., SCAE and SCHE at 400mg/kg b.w. and combination of TAAE (250mg/kg b.w.) and SCAE (400mg/kg b.w.) restored all the biochemical parameters towards the normal levels.

Histopathologically, the liver section of STZ induced diabetic rats showed necrosis and fibrotic changes with conspicuous evidence of fatty deposits, which got restored by extract of *Terminalia arjuna, Syzygium cumini* and their combinations treatment. The extract of *T. arjuna* restored the cellular arrangement in the central veins and decreased the necrosis (Ragavan and Krishnakumari, 2006). Ethanolic extract of *T. arjuna* showed the standard features in the liver of hyper-lipidemic rats (Shankreppa *et al.*, 2015) and the methanolic extract of S. cumini seeds-treated groups showed very favourable results when compared to standard group (Nahid *et al.*, 2017).

Kidney section of STZ induced diabetic rats showed necrosis and damage epithelium cells and proteinuria. At the same time, the extracts of *Heracleum persicum* reduced the degeneration and necrosis in the epithelium cells and deterioration of the glomeruli (Yaman *et al.*, 2017) which was similar to the extracts of *Terminalia arjuna, Syzygium cumini* and their combinations. Pancreas section of STZ induced diabetic rats showed damaged pancreatic islets cells, and the extract of *Nigella sativa* improved the morphological alteration such as necrotic and degenerative changes in the islets of Langerhans parenchyma (Mehmetkanter *et al.*, 2003) which was also similar to our findings.

CONCLUSION

Taken together, the present study for the first time reported the effect of *Terminalia arjuna* and *Syzygium cumini* (Alcoholic and Hydro-alcoholic) and their extract in STZ induced diabetes in wistar rats. Interestingly, improvement in body weight, glucose level, lipid profiles, biochemical parameters and histopathological changes in liver, kidney and pancreas was observed following herbal treatment in STZ induced diabetic rats. Furthermore, composite extract of TAAE (250mg/kg b.w) and SCAE (400mg/kg b.w.) was found to be more efficacious than the composite extract of TAHE (500mg/kg b.w.) and SCHE (400mg/kg b.w.).

ACKNOWLEDGEMENT

We are thankful to KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad for providing all the support to carry out the research work.

Conflict of interest

There is no conflict of interest among authors.

Funding Support

The authors have no funding support for this study.

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